Reference: *Biol. Bull.* 175: 410–416 (December, 1988)

Evolutionary Temperature Adaptation of Agonist Binding to the A_1 Adenosine Receptor

JOSEPH F. SIEBENALLER[1] AND THOMAS F. MURRAY[2]

[1]*Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803, and* [2]*College of Pharmacy, Oregon State University, Corvallis, Oregon 97331*

Abstract. The effects of temperature on the binding of the agonist [^{3}H]cyclohexyladenosine to A_1 adenosine receptors were studied by equilibrium binding techniques in brain membranes from eight vertebrate species with average body temperatures from 1 to 40°C. K_d values for rat and chicken increased markedly as measurement temperature decreased. In contrast, the K_d values for six teleost species, including warm-adapted, cold-adapted, and deep-living species, were much less sensitive to temperature perturbation. At 5°C K_d values vary 30-fold among the species; however, at temperatures approximating the cell temperatures of the species there is only a four-fold range of values. Binding enthalpies varied in sign and magnitude among the species. Binding entropies were positive for all the species; values were largest for the warm-adapted species, and smallest for the deep-living fishes. B_{max} values were relatively insensitive to temperature changes. $MgCl_2$ significantly increased B_{max} values, and for two of three species, lowered K_d values. $MgCl_2$ did not alter the enthalpy changes. In equilibrium competition experiments at 5°C using brain membranes from the deep-living teleost *Antimora rostrata*, the adenosine analogs R-phenylisopropyladenosine, N-ethylcarboxamidoadenosine, and 2-chloroadenosine were approximately 23-fold more potent than S-phenylisopropyladenosine. Despite perturbation by low temperature of agonist binding to mammalian and avian A_1 adenosine receptors, agonist recognition and binding properties of the A_1 receptor have been retained in vertebrates adapted to different body temperatures. These adaptive trends mirror those noted in studies of soluble enzyme homologs and muscle actins from species adapted to different temperatures.

Received 7 July 1988; accepted 27 September 1988.

Introduction

Adenosine has been demonstrated to be a significant endogenous modulator in mammalian tissues, and influences numerous physiological processes including lipolysis, coronary vasodilation, platelet aggregation, and neuronal function in the central nervous system (Snyder, 1985; Stiles, 1986; Williams, 1987). Adenosine modulates cyclic adenosine monophosphate (cAMP[1]) accumulation by affecting adenylate cyclase activity through two distinct membrane-associated receptors. At A_1 receptors, adenosine inhibits adenylate cyclase activity. At A_2 receptors, adenosine stimulates cAMP production (Daly *et al.*, 1981). These receptors are distinguished on the basis of structure-activity profiles of adenosine agonist analogs. The rank order potency of adenosine analogs is R-phenylisopropyladenosine (R-PIA) ≥ 2-chloroadenosine (2-CADO) ≥ N-ethylcarboxamidoadenosine (NECA) > S-phenylisopropyladenosine (S-PIA) at A_1 receptors and NECA > 2-CADO > R-PIA ≥ S-PIA at A_2 receptors (Daly, 1983 a,b; Stone, 1985; Williams, 1987).

A_1 adenosine receptors in central nervous tissue have a wide phylogenetic distribution among vertebrates (Siebenaller and Murray, 1986). Receptors were identified in brain membranes of eleven species representing six classes of vertebrates using the A_1-specific agonist [^{3}H]cyclohexyladenosine ([^{3}H]CHA) in assays at 22°C. Although all the vertebrates tested, including a number of cold-adapted marine fishes, displayed substantial amounts of specific [^{3}H]CHA binding, no specific bind-

[1] Abbreviations: 2-CADO, 2-chloroadenosine; cAMP, cyclic adenosine monophosphate; [^{3}H]CHA, [^{3}H]cyclohexyladenosine; EDTA, ethylenediaminetetraacetic acid; G protein, guanine nucleotide binding protein; NECA, N-ethylcarboxamidoadenosine; R-PIA, R-phenylisopropyladenosine; S-PIA, S-phenylisopropyladenosine; T_b, average body temperature.

ing could be detected in nervous tissue of molluscs or arthropods, the two invertebrate phyla tested (Siebenaller and Murray, 1986).

Ligand binding to the A_1 receptor is markedly sensitive to temperature perturbation (*e.g.*, Bruns *et al.*, 1980; Trost and Schwabe, 1981; Murphy and Snyder, 1982; Lohse *et al.*, 1984). In mammalian species, agonist binding is strongly perturbed by decreased temperatures (Murphy and Snyder, 1982); for example, optimal binding in rat adipocyte membranes is narrowly centered at 37°C (Trost and Schwabe, 1981). Agonist binding has a relatively large unfavorable enthalpy change and is strongly entropy-driven (Murphy and Snyder, 1982; Lohse *et al.*, 1984).

Because of the strong temperature dependence of agonist binding, and because ectothermic vertebrates have body temperatures spanning a broad range, a number of questions arise concerning A_1 adenosine receptors in ectotherms, particularly those living at cold temperatures. Do these receptors retain a high affinity for agonists at the average body temperature (T_b) of the species? Do A_1 receptors display the evolutionary conservation of ligand binding abilities documented for a variety of enzyme homologs in thermal adaptation? For example, in studies of homologs of M_4-lactate dehydrogenase from species with cell temperatures of −2 to 47°C, the K_m values of substrate and coenzyme are highly similar among species when measured at the T_b of the species, despite variation in K_m values with measurement temperature (Yancey and Somero, 1978; Graves and Somero, 1982; Hochachka and Somero, 1984; Yancey and Siebenaller, 1987). This selection for particular K_m values in evolutionary adaptation to temperature results in the preservation of the catalytic and regulatory functions of enzymes (Yancey and Somero, 1978; Graves and Somero, 1982; Hochachka and Somero, 1984; Siebenaller and Somero, 1988).

To elucidate the characteristics of A_1 adenosine receptors in evolutionary adaptation to temperature, a study was undertaken of the effects of temperature on binding of the A_1-specific agonist [^{3}H]CHA to brain membranes from species with body temperatures ranging from 1°C to 40°C, including a number of marine teleost fishes which differ in their depths of occurrence. [^{3}H]CHA was used as the ligand because of the problems associated with studying the binding of the endogenous agonist adenosine (Daly, 1983a).

Materials and Methods

Specimens

Demersal adult *Sebastolobus altivelis* and *S. alascanus* (Scorpaenidae) were taken by otter trawl at their typical depths of abundance off the coasts of Oregon and California. Demersal adult *Antimora rostrata* (Moridae), *Macrourus berglax* and *Coryphaenoides rupestris* (Macrouridae) were taken off the coast of Newfoundland, Canada. For comparisons these cold-adapted teleosts are grouped as shallow- or deep-living (Table I). Data on the depths of occurrence are from Wenner and Musick (1977), Sullivan and Somero (1980), Siebenaller *et al.* (1982), and Middleton and Musick (1986). Species which are common below approximately 600 m are considered deep-living. Brains were dissected and frozen in liquid nitrogen at sea and transported to the laboratory where they were stored at −80°C until used. Brains from *Epinephelus fulvus* (Serranidae) were provided by Dr. E. Pfeiler, University of Puerto Rico, Mayaguez, Puerto Rico, and shipped on dry ice. Frozen chicken (*Gallus domesticus*) and rat (*Rattus rattus*) brains were purchased from Pel-Freez Biologicals (Rogers, Arkansas). The T_b's of the species are given in Table I.

Chemicals

Radiolabeled [adenine-2,8-^{3}H]CHA (34.4 Ci/mmol) was purchased from DuPont NEN (Boston, Massachusetts). The R- and S-diastereomers of PIA and NECA were obtained from Research Biochemicals, Inc. (Wayland, Massachusetts). Adenosine deaminase (Sigma, Type VI), 2-CADO, and all other chemicals used were from Sigma Chemical Company (St. Louis, Missouri). Water was processed through a Milli-Q purification system (Millipore Corp., Bedford, Massachusetts).

Preparation of brain membranes

On the day of the experiment, frozen brain tissue was thawed and prepared in 50 m*M* Tris-HCl, pH 7.6 at 5°C, and 1 m*M* EDTA as described in Murray and Siebenaller (1987). Prior to the final resuspension of the tissue, the preparation was incubated with 2.5 IU/ml of adenosine deaminase at 20°C for 40 min. The final resuspension of the tissue was in 50 m*M* Tris-HCl, as indicated below.

Equilibrium binding assay for membrane bound A_1 adenosine receptors

The specific binding of the A_1-selective ligand [^{3}H]-CHA to brain membranes was determined using a rapid filtration assay described by Bruns *et al.* (1980) and Murray and Cheney (1982) with minor modifications. Assay conditions were chosen to be comparable to those used in a previous study of the two *Sebastolobus* species (Murray and Siebenaller, 1987).

Aliquots of brain membrane preparations (150–750 μg of protein) were incubated with [^{3}H]CHA and either buffer or competing compounds in a final volume of 1 ml. Samples were incubated in a circulating refrigerated

Table I

Thermodynamic parameters at 5°C and B_{max} values for [^{3}H]CHA binding to A_1 adenosine receptors in membranes from central nervous tissue of vertebrates with different body temperatures

Species	ΔG° (cal/mol)	ΔH° (cal/mol)	ΔS° (entropy units)	B_{max} (fmol/mg protein)
Warm-adapted				
Rattus rattus (37°C)[a]	9,474 ± 68[b]	9,259 ± 1,644*[c,d]	63.3	420 ± 18.6[e]
Gallus domesticus (40°C)	−10,195 ± 223	6,731 ± 1,365*	60.8	216 ± 7.9
Epinephelus fulvus (10 m; 24–25°C)	−10,556 ± 74	3,190 ± 1,013*	49.4	288 ± 9.8
Cold-adapted shallow-living teleost fishes				
Sebastolobus alascanus (180–440 m; 4–7°C)	−10,894 ± 6	−1,219 ± 422*	34.8	179 ± 4.0
Macrourus berglax (400 m; 1–4°C)	−11,075 ± 57	2,502 ± 845*	49.1	23 ± 0.9
Cold-adapted deep-living teleost fishes				
Sebastolobus altivelis (550–1200 m; 4–7°C)	−10,762 ± 37	212 ± 526	39.4	211 ± 8.9
Coryphaenoides rupestris (1100–1700 m; 1–6°C)	−10,634 ± 36	211 ± 809	39.0	71 ± 1.2
Antimora rostrata (1700 m; 1–4°C)	−10,405 ± 131	−3,773 ± 1,888	23.9	28 ± 2.9

[a] The range of body temperatures for the species, and for the marine fishes, the depth of occurrence are indicated.
[b] Mean of at least two determinations ± standard error.
[c] Calculated from the standard errors of the slopes of van't Hoff plots.
[d] * indicates ΔH° values not equal to zero ($P < 0.05$).
[e] Mean of determinations at all of temperatures ± standard error. The number of temperatures pooled for each species may be determined from Figure 1.

water bath (Model 2067, Forma Scientific, Marietta, Ohio) at the indicated temperatures for 2 h, since this time was found adequate in preliminary experiments for the binding reaction to achieve equilibrium at all temperatures. For each species except *Antimora rostrata*, experiments spanning the entire range of temperatures employed were performed in parallel to minimize variability in the treatment of the membrane preparations.

The binding reactions were terminated by filtration of the assay tube contents over No. 32 glass fiber filter strips (Schleicher and Schuell Inc., Keene, New Hampshire) using a cell harvester (model M-24R; Brandel Instruments, Gaithersburg, Maryland) under vacuum. Filters were then rinsed with four × 4-ml washes of ice-cold 50 m*M* Tris-HCl, pH 7.6 at 5°C, to remove unbound radioactivity. Filter disks were placed into counting vials to which was added 9 ml of Biocount (Research Products International Corp., Mount Prospect, Illinois). Filter-bound radioactivity was determined by liquid scintillation spectrometry (Beckman model LS8000) at an efficiency of 53% following overnight extraction at room temperature. The amount of radioligand bound was less than 10% of the total added in all experiments. Specific binding was defined as total binding minus binding occurring in the presence of 60 μ*M* R-PIA, and represented 95 to 85% of the total binding at the K_d values for [^{3}H]-CHA in all species.

Protein determination

Membrane protein content was assayed by the method of Lowry *et al.* (1951) following solubilization of the samples in 0.5 *M* NaOH. Bovine serum albumin (Sigma Chemical Company) was used as the standard.

Analysis

Each saturation binding isotherm of the agonist [^{3}H]-CHA to the A_1 receptor was analyzed using LUNDON-1 (Lundon Software, Inc., Cleveland, Ohio) iterative curve fitting routines (Lundeen and Gordon, 1985). The concentrations of [^{3}H]CHA used ranged from 0.08 n*M* to 24 n*M*.

Thermodynamic parameters were determined using the following equations:

$$K_a = 1/K_d$$

$$\Delta G^0 = -RT \ln K_a$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

where K_a is the equilibrium association constant, K_d the

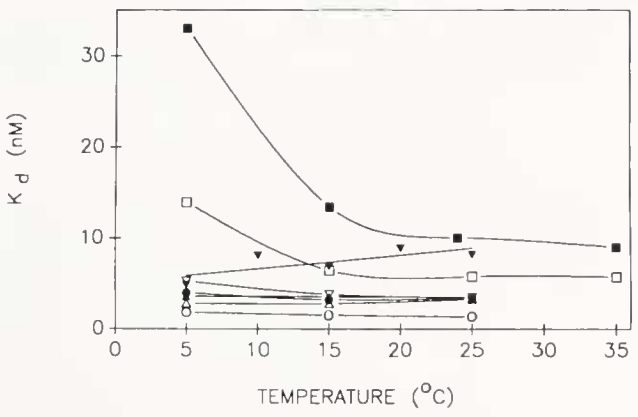

Figure 1. The effects of temperature on K_d of [^{3}H]CHA. Filled square: *Rattus rattus*, open square: *Gallus domesticus*; open inverse triangle: *Epinephelus fulvus*, open triangle: *Sebastolobus alascanus*, open circle: *Macrourus berglax*; filled triangle: *S. altivelis*, filled circle: *Coryphaenoides rupestris*; filled inverse triangle: *Antimora rostrata*. The standard error for each value is approximately 12% of the K_d. Each point represents at least two determinations.

apparent dissociation constant, ΔG^0 the standard free energy change, ΔH^0 the standard enthalpy change, ΔS^0 the standard entropy change, R the gas constant (1.987 cal mol^{-1} K^{-1}), and T the absolute temperature. Binding enthalpies were determined from van't Hoff plots of ln K_a *versus* the reciprocal absolute temperature using the integrated van't Hoff equation:

$$\ln K_a = -\Delta H^0/RT + \Delta S^0/R.$$

K_a values were calculated using the experimentally determined K_d at each temperature. The data were fit using a least squares linear regression.

For the [^{3}H]CHA competition experiments conducted at 5°C with *A. rostrata* brain membranes, IC_{50} values and slope factors were determined using the nonlinear least squares curve-fitting program LIGAND (Munson and Robard, 1980). Dissociation constants (K_i) were determined using the equation:

$$K_i = IC_{50}/[1 + ([L]/K_d)]$$

where [L] is the total [^{3}H]CHA concentration employed, K_d is the apparent dissociation constant of [^{3}H]CHA and IC_{50} is the concentration of inhibitor resulting in 50% inhibition of the specific binding (Cheng and Prusoff 1973).

Results

Effects of temperature on K_d and B_{max}

The effects of temperature at pH 7.6 on K_d of [^{3}H]-CHA values differed among the eight species studied (Fig. 1). Agonist binding in chicken and rat brain membranes was the most sensitive to temperature changes; K_d values increased 2- to 4-fold as temperature decreased from 35° to 5°C. In contrast, the K_d values for the six teleost fishes were much less sensitive to temperature; for five of these species, the K_d values changed less than 1.7 n*M* over the 20°C temperature range tested. The K_d values decreased with decreased measurement temperature for the preparations from *Antimora rostrata* and *Sebastolobus alascanus*. For *S. altivelis* and *Coryphaenoides rupestris* membranes, there were no changes in K_d over the temperature range 5–25°C. K_d values for *Epinephelus fulvus* and *Macrourus berglax* increased slightly with decreased temperature.

There is much less variation in K_d values at temperatures approximating the T_b's of the species (see Table I) than at an extreme measurement temperature such as 5°C. This is particularly apparent in comparing the K_d values of rat and chicken membranes at 35°C with the values for the cold-adapted fishes at 5°C (Fig. 1). There is a 30-fold range of values for all species at 5°C, and only a 4-fold range of K_d values at temperatures approximating the body temperatures of the species.

B_{max} values were relatively insensitive to temperature changes (*e.g.*, Fig. 3). The mean ± standard error of B_{max} values for all measurement temperatures pooled are presented in Table I.

Thermodynamic parameters

Binding enthalpies calculated from van't Hoff plots are given in Table I. A plot for representatives of the three groups of species is shown in Figure 2. The binding enthalpies differed among the species. The group of warm-adapted species, the rat, chicken, and *E. fulvus*, had the largest positive binding enthalpies. The two cold-adapted shallow-living fishes, *S. alascanus* and *M. berglax*, had

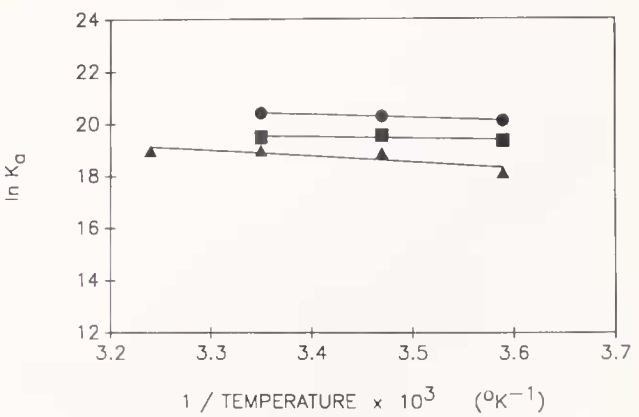

Figure 2. Van't Hoff plots for [^{3}H]CHA binding to brain membranes of *Coryphaenoides rupestris* (filled square); *Macrourus berglax* (filled circle); and *Gallus domesticus* (filled triangle).

Table II

Relative potencies of [illegible] analogs as inhibitors of specific [^{3}H]CHA binding [illegible] Antumora rostrata brain membranes

Analog	K_i (n*M*)	Hill slope
R-PIA	4.5 ± 0.96	0.94 ± 0.16
S-PIA	115.9 ± 47.98	0.77 ± 0.24
NECA	4.6 ± 1.20	1.06 ± 0.23
2-CADO	4.3 ± 1.68	0.75 ± 0.17

Ten concentrations of each analog were incubated with 3.8 n*M* [^{3}H]CHA.

enthalpy changes of different signs. The cold-adapted deep-living species had enthalpies which were close to zero or negative; the 95% confidence limits for the negative enthalpy of *A. rostrata* overlap zero.

ΔG^0 values were calculated using the K_d values obtained at 5°C. ΔS^0 was determined from ΔH^0 and ΔG^0 (Table I). The ΔS^0 values for all species were positive. The warm-adapted species had the largest binding entropies (49 to 63 cal/mol deg). The shallow-living, cold-adapted macrourid *M. berglax* had a large entropy change, similar to that of the warm-adapted serranid *E. fulvus* (49 cal/mol deg). The species with negative or zero binding enthalpies had the smallest entropy changes (24–39 cal/mol deg).

The membrane preparations from *C. rupestris* were additionally assayed in 50 m*M* imidazole-HCl, pH 7.5 at 20°C, and the pH allowed to vary with assay temperature. The pK_a of imidazole changes −0.017 pH units/°C, in a manner similar to the change in blood pH with temperature (Yancey and Somero, 1978; White and Somero, 1982). The change in enthalpy obtained in this regime of varying pH was similar to that obtained at a constant pH using Tris-HCl buffer, and the data were pooled.

Agonist equilibrium competition profile at 5°C

The pharmacological profiles of [^{3}H]CHA binding sites in *S. alascanus* and *S. altivelis* brain membranes at 22°C have been shown to be those expected for A_1 adenosine receptors, based on the affinities, rank order potencies, and ability to discriminate between the R- and S-diastereomers of PIA (Siebenaller and Murray, 1986; Murray and Siebenaller, 1987). To determine whether the pharmacological profile is similar at cold temperature in a cold-adapted species with temperature-dependent binding characteristics different from warm-adapted species, equilibrium competition experiments were performed using *A. rostrata* brain membranes at 5°C (Table II). The potencies of R-PIA, NECA, and 2-CADO were similar. R-PIA was approximately 23-fold more potent than S-PIA. The nanomolar affinities and the discrimination between R- and S-PIA are consistent with an A_1 adenosine receptor.

Effects of $MgCl_2$

For *C. rupestris*, *M. berglax*, and *G. domesticus*, the effects of 5 m*M* $MgCl_2$ were tested. Divalent cations have been shown to influence binding to A_1 receptors in mammalian tissue (Goodman *et al.*, 1982; Ukena *et al.*, 1984), and teleost nervous tissue (Murray and Siebenaller, 1987). B_{max} values in the three species increased substantially in the presence of 5 m*M* $MgCl_2$ at all temperatures and this increase tended to be greater at higher temperatures (Fig. 3). Although binding affinities increased in the presence of $MgCl_2$ in the chicken and *C. rupestris* membranes, the binding enthalpies in the presence and absence of $MgCl_2$ do not differ for any of the three species (*t*-test for each species $P > 0.05$) (data not shown).

Discussion

The affinity for agonists of the mammalian A_1 adenosine receptor is markedly decreased at lowered temperatures (Bruns *et al.*, 1980; Murphy and Snyder, 1982; Lohse *et al.*, 1984). The A_1 adenosine receptor can exist in two affinity states for agonists (Lohse *et al.*, 1984; Ukena *et al.*, 1984). The decreased affinity at low temperature is characteristic of the high-affinity state of the A_1 receptor, which comprises 70 to 85% of the receptor population in rat brain at high temperatures (Lohse *et al.*, 1984). In contrast, agonist binding to the low affinity state was exothermic, and the proportion of the receptors

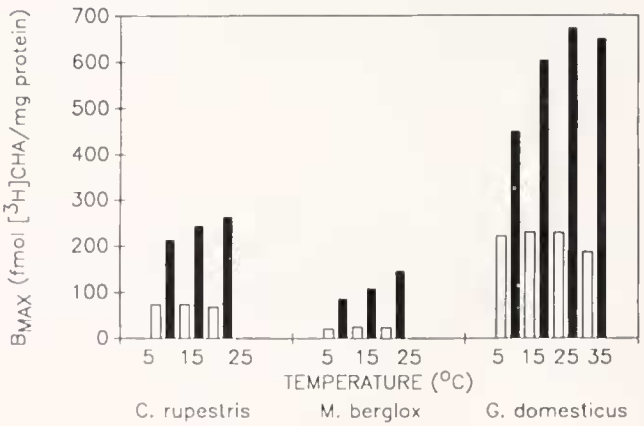

Figure 3. Effects of temperature and 5 m*M* $MgCl_2$ on B_{max} values of [^{3}H]CHA for *Coryphaenoides rupestris*, *Macrourus berglax*, and *Gallus domesticus* brain membranes. Open bars: no added $MgCl_2$; filled bars: 5 m*M* $MgCl_2$. Means of at least two experiments are shown; the standard error for each individual estimate of B_{max} is less than 9%; replicate estimates of B_{max} differ by less than 10%.

in the low affinity state increased at low temperatures (Lohse *et al.*, 1984).

The present study was undertaken to ascertain the pattern of temperature dependence of agonist binding to the A_1 receptor in vertebrates with different cell temperatures, particularly species with lowered body temperatures. With the protocol used, the data were consistently better fit by a one affinity state model (tested by a partial F-test, $P > 0.05$; see Hoyer *et al.*, 1984). Thus, this study cannot address potential temperature-dependent shifts among affinity states. Although the experimental protocol and incubation conditions of the present study differ in detail from that used in previous studies, the thermodynamic parameters obtained for rat (Table I) agree with those reported for mammalian nervous tissues by Murphy and Snyder (1982) (ΔH^0 13,200 cal/mol; ΔS^0 82 cal/mol deg at 25°C for CHA), and Lohse *et al.* (1984) for agonist binding to the high-affinity receptor subtype (ΔH^0 10,800 cal/mol; ΔS^0 76 cal/mol deg at 37°C for R-PIA binding).

Cold-adapted species retain high affinity binding at their T_b's, despite the strongly entropy-driven agonist binding displayed by such warm adapted species as the rat and chicken (Fig. 1; Table I). The affinity of agonist binding to the A_1 receptor in nervous tissue of cold-adapted ectotherms at their T_b's is comparable to that displayed by warm-adapted species at high temperatures. Although there is considerable variation in K_d values at different measurement temperatures, this variation is greatly reduced when comparisons are made at temperatures approximating the T_b's of the species. This contrast is most apparent when one compares the 30-fold range of values at 5°C to the 4-fold range of K_d values at temperatures approximating the cell temperatures of the species (Fig. 1; Table I).

$MgCl_2$ significantly increased B_{max} values (Fig. 3), and for the chicken and *C. rupestris* increased binding affinities. The binding enthalpies were not altered by $MgCl_2$; the Mg^{2+}-stimulated receptors have temperature-dependencies similar to the receptors treated with EDTA and incubated in the absence of $MgCl_2$.

The study at 5°C of *A. rostrata* (Table II) and previous studies of the *Sebastolobus* species (Siebenaller and Murray, 1986; Murray and Siebenaller, 1987) indicate that the pharmacological profile of A_1 receptors of deep-sea and cold-adapted fishes are comparable to those of mammals in terms of their affinities and in the discrimination of the R- and S-diastereomers of PIA, even when the receptors of a cold-adapted species are assayed at 5°C.

The A_1 receptors in vertebrates with widely different T_b's (1–40°C) have similar binding affinities for [^{3}H]-CHA at temperatures approximating the T_b's of the species. This is analogous to the evolutionary conservation of K_m values for enzyme homologs from species with different T_b's. At the T_b's of the species K_m values are highly similar, even though there is a strong temperature-dependence of substrate and coenzyme binding (Yancey and Somero, 1978; Graves and Somero, 1982; Hochachka and Somero, 1984; Yancey and Siebenaller, 1987). Conservation of K_m values, coupled with the similar *in vivo* substrate levels among species, results in the preservation of the regulatory and catalytic properties of the enzymes, and the sparing of cellular solvent capacity (*e.g.*, discussion in Hochachka and Somero, 1984).

The conservation of ligand recognition and binding affinity among receptors in species with different T_b's reflects the preservation during evolution of the interactions between agonist and receptor. Ligand binding to a receptor, such as the A_1 adenosine receptor which is coupled to adenylate cyclase, is but a single step in the function of the receptor—G protein—adenylate cyclase circuitry. Nonetheless, ligand recognition and binding are critical in the transmembrane signaling process.

The relative roles of enthalpy and entropy changes in establishing the net change in free energy vary systematically among species with different body temperatures (Table I) in a manner mirroring the adaptive changes in binding observed for actins from vertebrates with different body temperatures (Swezey and Somero, 1982). The differences among species in thermodynamic characteristics of agonist binding to the A_1 receptors may reflect a variety of factors which have yet to be evaluated. These factors may include differences (1) in the primary structure of the receptors, (2) in the carbohydrate moieties covalently bound to the receptor proteins, (3) in the lipid milieu of the membranes, and (4) in the interaction of the receptor with the inhibitory G protein (G_i), either in terms of the number and types of weak bonds involved in receptor protein-G_i binding, or in the relative quantities of G_i and A_1 receptor. It is likely that all of these may contribute to the adjustment of binding affinities with temperature, and indeed, in different evolutionary lineages, the relative importance of these mechanisms may differ.

Acknowledgments

This work was supported by NSF grants DCB-8416602 and DCB-8710155. Ship time on the R/V *Wecoma* cruises off the coast of Oregon were supported by these grants. Ship time on the R/V *Gyre* off the coast of Newfoundland was supported by NSF grant DMB-8502857 to Dr. A. F. Riggs. We thank Drs. A. Gibbs, E. Pfeiler, A. Riggs, and R. Noble for their help in obtaining specimens.

Literature Cited

Bruns, R. F., J. W. Daly, and S. H. Snyder. 1980. Adenosine receptors in brain membranes: Binding of N^6-cyclohexyl[^{3}H]adenosine

and 1,3-diethyl-8-[^{3}H]phenylxanthine. *Proc. Natl. Acad. Sci. USA* **77:** 5547–5551.

Cheng, Y. C., and W. H. Prusoff. 1973. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22:** 3099–3108.

Daly, J. W. 1983a. Adenosine receptors: characterization with radioactive ligands. Pp. 59–69 in *Physiology and Pharmacology of Adenosine Derivatives*, J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu, and M. Ui, eds. Raven, New York.

Daly, J. W. 1983b. Role of ATP and adenosine receptors in physiological processes: Summary and prospectus. Pp. 275–290 in *Physiology and Pharmacology of Adenosine Derivatives*, J. W. Daly, Y. Kuroda, J. W. Phillis, H. Shimizu, and M. Ui, eds. Raven, New York.

Daly, J. W., R. F. Bruns, and S. H. Snyder. 1981. Adenosine receptors in the central nervous system: Relationship to the central action of methylxanthines. *Life Sci.* **28:** 2083–2097.

Goodman, R. R., M. J. Cooper, M. Gavish, and S. H. Snyder. 1982. Guanine nucleotide and cation regulation of the binding of [^{3}H]cyclohexyladenosine and [^{3}H]diethylphenylxanthine to adenosine A_1 receptors in brain membranes. *Mol. Pharmacol.* **21:** 329–335.

Graves, J. E., and G. N. Somero. 1982. Electrophoretic and functional enzymic evolution in four species of eastern Pacific barracudas from different thermal environments. *Evolution* **36:** 97–106.

Hochachka, P. W., and G. N. Somero. 1984. *Biochemical Adaptation.* Princeton University Press, Princeton, New Jersey.

Hoyer, M. M., E. E. Reynolds, and P. B. Molinoff. 1984. Agonist-induced changes in the properties of *beta*-adrenergic receptors on intact S49 lymphoma cells. Time-dependent changes in the affinity of the receptor for agonists. *Mol. Pharmacol.* **25:** 209–218.

Lohse, M. J., V. Lenschow, and U. Schwabe. 1984. Two affinity states of R_i adenosine receptors in brain membranes. Analysis of guanine nucleotide and temperature effects on radioligand binding. *Mol. Pharmacol.* **26:** 1–9.

Lundeen, J. E., and J. H. Gordon. 1985. Computer analysis of binding data. Pp. 31–49 in *Receptor Binding in Drug Research*, R. O'Brien, ed. Marcel Dekker, New York.

Middleton, R. W., and J. A. Musick. 1986. The abundance and distribution of the family Macrouridae (Pisces: Gadiformes) in the Norfolk Canyon area. *Fish. Bull.* **84:** 35–62.

Munson, P. J., and D. Rodbard. 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107:** 220–239.

Murphy, K. M. M., and S. H. Snyder. 1982. Heterogeneity of adenosine A_1 receptor binding in tissue. *Mol. Pharmacol.* **22:** 250–257.

Murray, J. F., and D. L. Cheney. 1982. Localization of N^6-cyclohexyl[^{3}H]adenosine binding sites in rat and guinea pig brain. *Neuropharmacology* **21:** 575–580.

Murray, J. F., and J. F. Siebenaller. 1987. Comparison of the binding properties of A_1 adenosine receptors in brain membranes of two congeneric marine fishes living at different depths. *J. Comp. Physiol. B* **157:** 267–277.

Siebenaller, J. F., and J. F. Murray. 1986. Phylogenetic distribution of [^{3}H]cyclohexyladenosine binding sites in nervous tissue. *Biochem. Biophys. Res. Commun.* **137:** 182–189.

Siebenaller, J. F., and G. N. Somero. 1988. Biochemical adaptation to the deep sea. *Crit. Rev. Mar. Sci.* **1:** in press.

Siebenaller, J. F., G. N. Somero, and R. L. Haedrich. 1982. Biochemical characteristics of macrourid fishes differing in their depths of distribution. *Biol. Bull.* **163:** 240–249.

Snyder, S. H. 1985. Adenosine as a neuromodulator. *Ann. Rev. Neurosci.* **8:** 103–124.

Stiles, G. L. 1986. Adenosine receptors: structure, function and regulation. *Trends Pharmacol. Sci.* **7:** 486–490.

Stone, T. W. 1985. Summary of a symposium on purine receptor nomenclature. Pp. 1–5 in *Purines. Pharmacology and Physiological Roles*, T. W. Stone, ed. UCH Publishers, Deerfield Beach, Florida.

Sullivan, K. M., and G. N. Somero. 1980. Enzyme activities of fish skeletal muscle and brain as influenced by depth of occurrence and habits of feeding and locomotion. *Mar. Biol.* **60:** 91–99.

Swezey, R. R., and G. N. Somero. 1982. Polymerization thermodynamics and structural stabilities of skeletal muscle actins from vertebrates adapted to different temperatures and pressures. *Biochemistry* **21:** 4496–4503.

Trost, T., and U. Schwabe. 1981. Adenosine receptors in fat cells. Identification by (-)-N^6-[^{3}H]phenylisopropyladenosine binding. *Mol. Pharmacol.* **19:** 228–235.

Ukena, D., E. Poeschla, and U. Schwabe. 1984. Guanine nucleotide and cation regulation of radioligand binding to R_i adenosine receptors of rat fat cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **326:** 241–247.

Wenner, C. A., and J. A. Musick. 1977. Biology of the morid fish, *Antimora rostrata*, in the western North Atlantic. *J. Fish. Res. Board Can.* **34:** 2362–2368.

White, F. N., and G. N. Somero. 1982. Acid-base regulation and phospholipid adaptations to temperature: time courses and physiological significance of modifying the milieu for protein function. *Physiol. Rev.* **62:** 40–90.

Williams, M. 1987. Purine receptors in mammalian tissues: pharmacology and functional significance. *Ann. Rev. Pharmacol. Toxicol.* **27:** 315–345.

Yancey, P. H., and J. F. Siebenaller. 1987. Coenzyme binding ability of homologs of M_4-lactate dehydrogenase in temperature adaptation. *Biochim. Biophys. Acta* **924:** 483–491.

Yancey, P. H., and G. N. Somero. 1978. Temperature dependence of intracellular pH: its role in the conservation of pyruvate apparent K_m values of vertebrate lactate dehydrogenases. *J. Comp. Physiol.* **125:** 129–134.